GRAPES, GALLS, AND GEOGRAPHY: THE DISTRIBUTION OF NUCLEAR AND MITOCHONDRIAL DNA VARIATION ACROSS HOST-PLANT SPECIES AND REGIONS IN A SPECIALIST HERBIVORE

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Abstract.—Studies of patterns of molecular variation in natural populations can provide important insights into a number of evolutionary problems. Among these, the question of whether geographic factors are more important than ecological factors in promoting population differentiation and ultimately speciation has been an important and contentious area in evolutionary biology. Systems involving herbivorous insects have played a leading role in this discussion. This study examined the distribution of molecular variation in a highly specialized gall-forming insect, grape phylloxera (*Daktulosphaira vitifoliae* Fitch), that is found on both sympatric and allopatric host-plant species of the genus *Vitis*. In addition, the relationship of insects in the introduced range in the United States to ancestral populations in the native range was examined. Evidence for differentiation along host-plant lines from both nuclear (RAPD) and mitochondrial (COI) DNA was confounded with the effect of geography. Differentiation was found where hosts were allopatric or parapatric, but no evidence was found for such differentiation on two hosts, *V. vulpina* and *V. aestivalis,* that are broadly sympatric. The question of population differentiation onto these sympatric hosts can be considered—it has not occurred in spite of a long history of association. Evidence was equivocal, but suggestive of a period of divergence in allopatry prior to reestablishment of contact, for insects associated with another host plant species, *V. cinerea,* found in both sympatric and parapatric populations. A low level of diversity and placement of samples collected from the grape species *V. riparia* at the tip of a phylogenetic tree supports the hypothesis that this host has been recently colonized from populations from the Mississippi Valley. A polyphyletic origin for biotype B grape phylloxera was supported: Although most samples collected from vineyards in the introduced range in California had similar haplotypes, they were closely related to natives on *V. vulpina* from the Atlantic Coast–Piedmont region. All samples collected from vineyards in Oregon and Washington were closely related to natives on *V. riparia* in the northern United States.

Key words.—Biotypes, cytochrome oxidase I, geographic distribution, grape phylloxera, herbivorous insects, host-plant variation, sympatric divergence, *Vitis.*

Geographical features of the environment of organisms that prevent or reduce gene flow are widely considered to be better predictors of genetic differentiation of populations than are ecological factors (Dobzansky 1937; Mayr 1963; Futuyma and Mayer 1980; Patterson 1981; Templeton 1981; Carson 1982; Barton and Charlesworth 1984; Lynch 1989; Allmon 1992; Avise 2000; Barraclough and Vogler 2000), but the consensus on this issue is far from complete. Increasing theoretical support for the sympatric model of speciation has accumulated over the years and many evolutionary biologists now feel quite comfortable with this model (Maynard Smith 1966; Bush 1969; Rosenzweig 1978; Kondrashov and Mina 1986; Rice 1987; Diehl and Bush 1989; Kawecki 1996; Schluter 1996; see reviews in Howard and Berlocher 1998). Herbivorous insects in particular have been extolled as likely candidates to demonstrate the potential for ecological heterogeneity to promote genetic diversity and divergence (Walsh 1864; Bush 1969, 1993; Menken 1981; Wood and Guttman 1983; Tauber and Tauber 1989; Johnson et al. 1996; Abrahamson and Weis 1997). This focus arises from the fact that for many herbivorous insects the host plant is home and mating site, as well as sole resource. The consequences of this tight specialization could be linkage between host choice, mating, and performance and a reduction in gene flow among populations across divergent host plants. A hypothesis of herbivore diversification driven by divergent host plants therefore predicts a host-associated distribution of genetic variation.

In herbivorous insects with broad geographical ranges the pattern of ecological divergence will be overlaid onto a pattern due to spatial disjunction and isolation by distance. If a host-associated population arose in a localized area and subsequently spread, geographic structure may appear within a host-specific clade. Paraphyly may be expected where there is continued gene flow (expected to be reduced or absent in host-associated races or species), renewed gene flow after secondary contact, or under incomplete lineage sorting that may be prolonged with low levels of gene flow or if divergence onto a specific host occurs in already geographically structured populations (Wakeley 2000). It is difficult to distinguish between these possibilities, but the behavior of different classes of markers may provide insight. If divergent host plants are not sympatric, attempts to relate genetic divergence among herbivore populations to ecological causes in the form of host-plant variation will be confounded. To show that host-plant variation has driven divergence among herbivore populations, such divergence must be found where different hosts are sympatric. Demonstrating sympatric divergence constitutes necessary but not sufficient evidence for host-plant-driven differentiation because host-associated populations may have come into contact secondarily. As pointed out by Berlocher (1998), in spite of the attention

given to them, there are few convincing cases of sympatric speciation in herbivorous insects. Despite these difficulties and those inherent to the study of natural populations where the replicated, balanced designs that can be set up in a laboratory may not be available, examining patterns of molecular variation may still provide insight into factors that drive divergence.

Many pest species in crop systems are introduced onto agricultural hosts from related native hosts in unmanaged systems. The adaptive range of these herbivores has been shaped by the evolutionary history of association with their native hosts. Unfortunately, that history is usually, at best, poorly known. The pattern of genetic variation in herbivore populations within and among host plants and geographic regions in the source populations and how this variation has been sampled by introductions into agroecosystems will play a large role in how pest populations respond to the selective pressures imposed by management efforts. Because introductions into agriculture have occurred recently, the pest populations should show little molecular divergence from their nearest relatives on wild host plants. In addition, when confronted with insects able to survive and reproduce on crop cultivars that have previously been considered resistant, entomologists often invoke the biotype concept to categorize these insects (Saxena and Barrion 1987). Biotypes are classified on the basis of performance on different resistant host plants and have been explicitly equated with host races, and this meaning is implicit in most usages of the term (Diehl and Bush 1984; Saxena and Barrion 1987), although the entity being categorized is otherwise often poorly defined. Biotype's origins and status as evolutionary lineages are relevant to researchers interested in population differentiation and genetic structure. There are two hypotheses for the origins of host-associated pest populations (biotypes). First, a single introduction may have occurred or single source location may exist, with subsequent adaptive divergence. Little neutral molecular differentiation between genotypes associated with different hosts is predicted because the time has been too short for much sequence divergence to accumulate. Second, multiple, independent introductions may have occurred. Two possibilities for this case are: (1) phenotypic convergence of genotypes from different sources, which could result from response to selective pressure in the agroecosystem, or introduction of preadapted genotypes; host associated pest populations will be polyphyletic; or (2) introductions from separate and adaptively divergent native populations; pest populations associated with different hosts will be monophyletic.

Here we focused on a galling insect that specializes on grapes (*Vitis* spp.) and is widely introduced into agroecosystems, the grape phylloxera, *Daktulosphaira vitifoliae* Fitch. Grape phylloxera exhibits many of the characteristics thought to promote host-plant-associated genetic divergence, such as linkage of important life-history events (including mating) with its host plant, host choice and performance linkage (Omer at al. 1999), and genetic variation for fitness on different hosts (De Benedicits and Granett 1992; Fergusson-Kolmes and Dennehy 1993; Hawthorne and Via 1994; Downie 1999a). It should be a likely candidate for a sympatric host-driven model of genetic divergence. Previously, geographic differentiation for random amplified polymorphic

DNA (RAPD) and amplified fragment length polymormorphism (AFLP) markers has been found (Lin et al. 1999; Downie and Granett 2000; H. Lin., M. A. Walker and D. A. Downie, unpubl. data), but sampling has not been comprehensive. The scale of the Mendelian population in the continuous habitat of the eastern United States is not known, but may be large. Downie (2000) found no isolation by distance and little differentiation for RAPD markers across two sympatric host-plant species at the scale of a 145-km transect, suggesting that a focus on broad scale, rather than local population level patterns, was appropriate. The present study thus scales up to include most of the native range and a portion of the introduced range.

The grape phylloxera–*Vitis* system provides an opportunity to examine the relative roles of geography and ecological heterogeneity in fostering genetic divergence and diversity and to trace the ancestry of populations in an introduced range of an important pest species. The opportunity for linkage between applied and basic interests make the grape phylloxera–*Vitis* relationship attractive for evolutionary biologists seeking to narrow the gap between disciplines. In addition, more data from more systems are needed for a general understanding of the determinants of genetic structure, and this work helps to achieve that need.

Here we used a phenetic approach (RAPD) to survey multiple unlinked nuclear loci and a phylogenetic approach by sequencing a region of mitochondrial DNA to address two main questions: How is molecular variation distributed across host-plant species and geographic regions? What are the evolutionary relationships within and among native and pest populations?

MATERIALS AND METHODS

The System

Grape phylloxera is native to North America and is currently considered the single species of a monotypic genus, but study of its ecology and evolution in its native range has been sparse. In the generalized life cycle, the hatching of overwintering zygotic eggs in the spring produces fundatrices that create pouch galls on newly forming leaves. They spend the rest of their lives in the galls and lay eggs by ameiotic parthenogenesis (Morgan 1909; Davidson and Nougaret 1921; Maillet 1957; Forneck et al. 1999; Lin et al. 1999). Adults are less than 1 mm in length (Davidson and Nougaret 1921). A variable number of parthenogenetic generations ensue, with first instars (crawlers) of each generation forming galls on newly forming leaves. As the season progresses, some crawlers move to roots, where they attach themselves and create galls primarily on new rootlets. Winged morphs (alatae) produced by the root-galling morphs migrate to the canopy of new or of the same hosts, where they lay eggs of sexual (sexuales) males and females. Sexuales spend all but their final molt in an immobile pupiform state (Stoetzel 1985). They are without mouthparts and so do not feed. At maturity they live a few days, during which mating occurs. After mating, each female lays a single diapausing egg. Other than dispersal of sexuales by alates, movement among host plants must be accomplished by crawlers walking or being blown by wind (Hawthorne and Dennehy 1991). This com-

TABLE 1. Grape phylloxera life-cycle traits and population size measured by relative abundance across host-plant species and geographic regions. Estimates for N_e are not available. Sexuparae are either alate morphs produced by root-feeding individuals or apterous leaf-galling individuals and are the mothers of sexuales. *Vitis girdiana* has only been studied in two locations near Death Valley, California. Although alate sexuparae are found on the Pacific Coast of the United States, they apparently do not produce viable sexuales. See Figure 2 for definition of geographic regions.

Host/region	Leaf galling	Root galling	Sexuparae	Population size
V. vulpina/GC, CE, ACP, NE	+	+	alates	large, except ACP, GC
V. aestivalis/GC, CE, ACP, NE	+	+	alates	small
V. cinerea/GC, CE, ACP	+	−[1]	apterous	small
V. riparia/NE, NC	+	+	alates	large
V. arizonica/SW	+	−	apterous	moderate
V. girdiana/SW	+	−	apterous	small
Cultivars/PC	−	+	alates	large

[1] The absence of root galling is assumed because sexuales are produced directly from leaf-galling individuals.

plex life cycle has been subject to truncations and morph shifts in some regions and on some hosts (Riley 1876; Davidson and Nougaret 1921; Downie and Granett 1998; Downie et al. 2000; see below and Table 1).

Grape phylloxera is distributed on wild grapes across the United States east of the Rocky Mountains and into the southwestern United States and Mexico; current data document six of the some 18 North American *Vitis* species (Moore 1991) comprising the native host range. The distribution is disjunct between southwestern and eastern populations (Downie and Granett 1999; Downie et al. 2000), so that some indigenous *Vitis* host species are allopatric, but a large region of sympatry occurs from north of the Ohio and Missouri Rivers to the Gulf Coastal Plain (Fig. 1). In the southwest

United States and Mexico, *V. arizonica* Englemann is attacked by leaf-galling phylloxera only (Downie and Granett 1998). This appears to be true for *V. girdiana* Munson as well, although only two sites in southeastern California have been studied (Granett et al. 1991). The loss of root-galling alate morphs and the production of sexuales by leaf-galling females mean that mating on the natal host is likely. Recent evidence suggests this life-cycle variation is shared by grape phylloxera on *V. cinerea* Engelmann (Downie et al. 2000). This species is sympatric across the central and eastern United States with two other species (*Vitis vulpina* L. and *V. aestivalis* Michaux) that differ in morphological, biochemical, and phenological traits (Bailey 1934; Moore 1991; Moore and Giannasi 1994; J. Grinstead, pers. comm.). *Vitis vulpina*
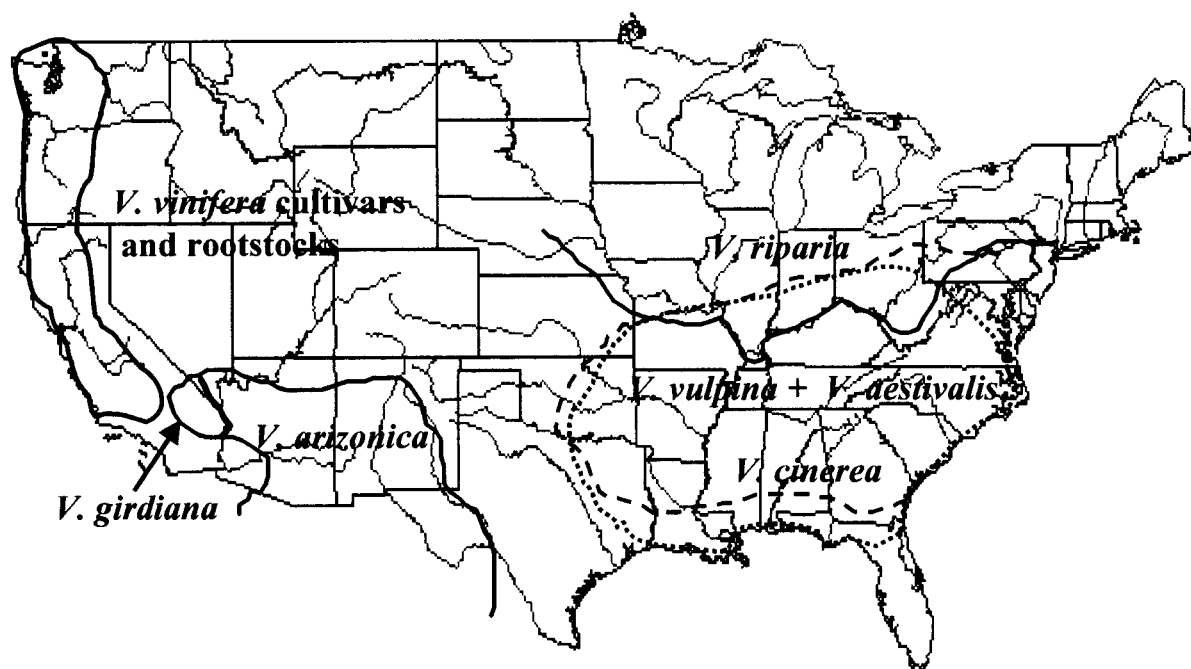

FIG. 1. Map showing the distribution of *Vitis* species known to host grape phylloxera. *V. vinifera* cultivars include own-rooted *V. vinifera* L. vines as well as rootstocks that support grape phylloxera to varying extent, in particular AXR 1 (*V. rupestris* × *V. vinifera*), but also Harmony (complex hybrid), 3309C (*V. riparia* × *V. rupestris*), 110R (*V. berlandieri* × *V. rupestris*), and 5C (*V. berlandieri* × *V. riparia*). Cultivars of *V. vinifera* as well as other grape species and their cultivars are grown in vineyards and attacked by phylloxera elsewhere in the United States, particularly in New York State. The distribution of *V. cinerea* is marked by short dashes and the distributions of *V. vulpina* and *V. aestivalis* are marked by long dashes. Distribution data were taken primarily from Downie and Granett (1998, 1999) and Downie et al. (2000).

and *V. aestivalis* are more common toward the north, whereas *V. cinerea* is more common to the south and is the predominant host along the Gulf Coast. Throughout the formerly glaciated region north of the Ohio and Missouri Rivers, the species *V. riparia* Michaux is the dominant or sole grape species present. The highest frequency of attack, and by inference the largest population, has been found in this region (Downie et al. 2000).

Through transport of infested grapevine material used in breeding and propagation, the insect has been introduced and thrives on cultivars of the wine grape, *V. vinifera* L., in viticultural regions throughout the world, with the possible exceptions of Chile and southern Australia. On the Pacific Coast of the United States, the insect is found primarily on the roots of infested plants, where it creates galls on hardened roots as well as on newer rootlets. The life cycle is thought to be completely parthenogenetic (anholocyclic). Host-plant resistance has been used to control the insect by grafting *V. vinifera* scions onto rootstocks selected or bred from native North American grapes, although phylloxera genotypes exist that flourish on some of these, notably the hybrid AXR 1 (*V. rupestris* Scheele × *V. vinifera*). Genotypes with high fitness on rootstock AXR 1 (biotype B) or *V. vinifera* (biotype A) have been named as biotypes in California (Granett et al. 1983, 1985), but evidence from molecular markers has suggested that biotypes are heterogeneous (Fong et al. 1995). In any case, genetic variation in performance on different hosts clearly exists.

Sampling

Grape phylloxera were collected from wild grapes in 24 states in the continental United States. Sampling was conducted in 1997 in the Southwest (Arizona and New Mexico) and in 1999 in the central and eastern United States. Samples were collected by taking galled shoots from infested grapevines and holding these at about 10°C until dissecting galls in the laboratory. Where possible, eggs from a single adult formed a sample. In some cases, to ensure enough material for all experiments, eggs as well as adults from multiple galls were pooled to form a sample. In such cases, galls were located in close proximity to increase the probability of sampling a single clone. An effort was made to sample across the native range in a grid pattern, with a single sample taken at randomly chosen locations with an average separation of 130 km (± 73 km SD). The focus was on broad-scale patterns under the premise that a meaningful conception of host-associated races or species in this system, when there is no evidence of a very recent host shift, suggests a reasonably broad distribution. The aim was to maximize the number of local populations (demes) sampled, rather than the number of samples within a small number of widely spaced local populations. The sampling scheme thus reflects a trade-off between local sampling intensity and geographic coverage. Given the same geographic coverage, replication within local populations would mean that total number of samples would have to be increased by a factor of at least six, for which resources were not available. Given the same sampling effort, a small number of widely separated populations would be sampled, leaving many intervening populations unsampled.

This leads to the potential for an artifactual geographic structuring due to the correlation among individuals within a local mating population and isolation by distance among widely separated populations. The current scheme was chosen to minimize this potential artifactual structure while still effectively testing the hypothesis that host-associated races or species exist. Note that all comparisons are therefore at the level of the host-plant species or geographic region (as defined here), not among sampling sites. The units of replication are the host-plant species and the regional population.

Grape phylloxera were also sampled from roots in vineyards from cultivated grapes in California, Oregon, and Washington in 1998 and 1999, and from leaf galls in two vineyards in New York State in 1999. Samples from roots were collected by digging approximately 24 cm³ around the trunks of vines and clipping infested roots from vines. Colonies from these collections were established in the laboratory by inoculating eggs onto excised root pieces of *V. vinifera* cultivar Merlot, as in Granett et al. (1983). After establishment, these colonies were passed through a bottleneck of a single individual to establish a clone. Eggs were collected from a given clone to form a sample for DNA extraction and molecular experiments. Single clones from each of 22 vineyards in the four states were used in analyses.

For use as outgroups, two related species of Phylloxeridae were collected, a *Phylloxera* species from hickory (*Carya* sp.) in Cape Fear, North Carolina, and a *Phylloxera* species from oak (*Quercus lobata* Nee) in Davis, California. The basal position of oak phylloxera relative to grape phylloxera is inferred by the absence of the galling habit and a distribution spanning the Northern Hemisphere, whereas grape phylloxera is of North American origin. Phylloxera on hickory have diversified intensively (~ 50 species), suggesting an older history than grape phylloxera, although the radiation may have occurred since the split leading to grape phylloxera. Sampling locations and geographic regions as defined in Downie et al. (2000) and their abbreviations as used below are shown in Figure 2. Additional sampling details are given in the Appendix.

DNA Extraction, Polymerase Chain Reaction, and Sequencing

DNA extraction followed the protocol of Lin and Walker (1996) and Downie (2000), with the modification that samples were ground in a 0.6-ml tube with a Teflon pestle (Kontes Scientific Glassware, Vineland, NJ). All DNA samples were quantified by spectrophotometry and diluted to 10 ng/μl.

For RAPD analysis, seven primers were used (Operon primers OPG5, OPG9, OPG10, OPG12, OPG14, OPG16, OPG18; Operon Technologies, Alameda, CA). The reaction components and final concentrations were polymerase chain reaction (PCR) buffer (50 mM Tris-HCL [pH 8.0], 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 50% glycerol, 0.5% Tween, 1% Triton X-100; Promega Biotech, Madison, WI), 3 mM MgCl$_2$, 0.2 mM dNTPs, 0.4 μM primer, 1 unit *Taq* polymerase (Promega Biotech), and 30 ng DNA template to a final volume of 20 μl with sterile ddH$_2$O. The PCR cycling profile was: 5 min at 94°C followed by 4 cycles of 94°C for 30 sec, 36°C for 1 min, and 72°C for 2 min; then 35 cycles
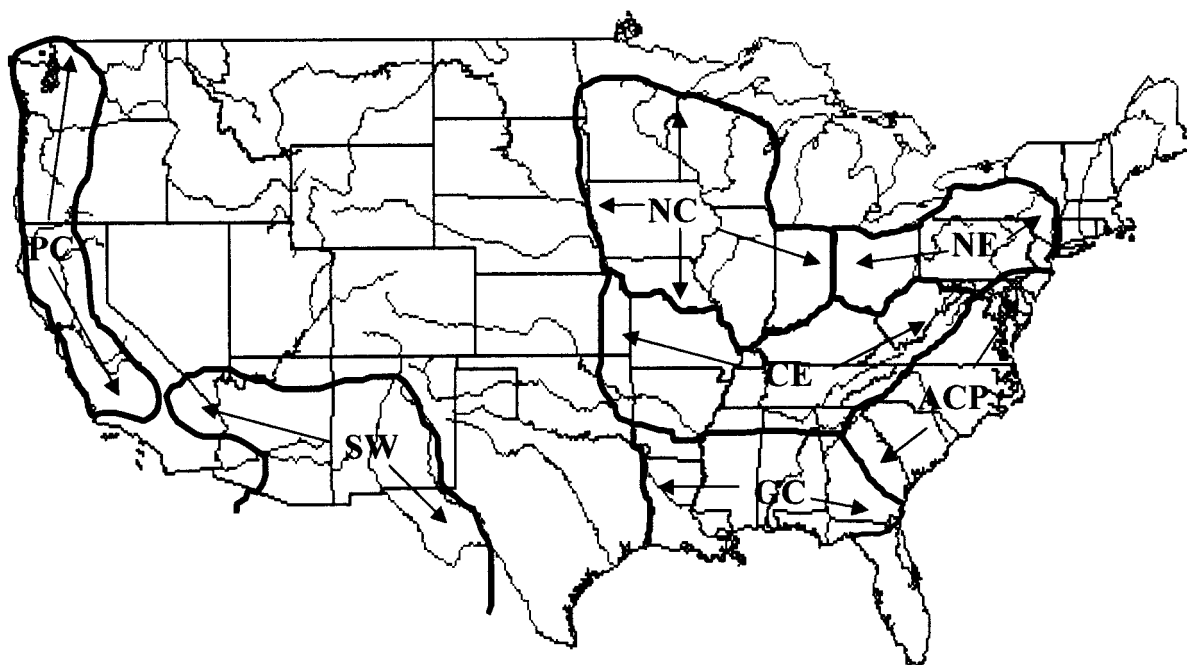
FIG. 2. Map showing geographic regions as defined in Downie et al. (2000) and used for heuristic purposes in discussing the geographic distribution of grape phylloxera and its host plants. PC, Pacific Coast; SW, Southwest; GC, Gulf Coast; CE, Central; ACP, Atlantic Coast–Piedmont; NE, Northeast, NC, North-central.

of 94°C for 10 sec, 36°C for 30 sec, and 72°C for 1 min; and a final extension of 72°C for 5 min. Reactions were run on either a Perkin-Elmer 2400 or a Perkin-Elmer 9700 thermalcycler (Perkin-Elmer Biosystems, Foster City, CA).

PCR products were separated on 1.5% agarose gels (FMC BioProducts, Rockland, ME) and visualized on a UV illuminator after ethidium bromide staining. Each gel contained a random sample of the entire set of samples and included a negative control (no DNA template). Samples were coded without reference to host plant or geographic region of collection so that scoring of gels was blind. Codes were matched to collection data in a separate master list that was referred to after scoring.

A region toward the 5′ end of the cytochrome oxidase I (COI) gene was amplified using primers C1-J-1718 (5′-GGAGGATTTGGAAATTGATTAGTTC-3′) and C1-N-2191 (5′-CCCGGTAAAATTAAAATATAAACTTC-3′) found in Simon et al. (1994) and corresponding to a 473-bp fragment in the *Drosophila yakuba* sequence. Reaction components and final concentrations were as above except for 2 mM $MgCl_2$, 0.2 μM each primer, and 40 ng DNA template in a final volume of 25 μl. Cycling parameters were: 2 min at 94°C, followed by 40 cycles of 30 sec at 94°C, 1 min at 52°C, and 2 min at 72°C. Some samples from the Southwest gave better amplification products at an annealing temperature of 48°C. PCR products were purified with Microcon-100 microconcentrators (Amicon, Beverly, MA). Ninety-two samples were sequenced, on both strands. All sequencing was done at the Department of Biological Sciences Automated Sequencing Facility at the University of California, Davis, using the ABI Big Dye terminator sequencing reaction kit (Perkin-Elmer/ABI, Weiterstadt, Germany) on an ABI Prism 377 automatic sequencer with XL upgrade (Perkin-Elmer) on 5% acryl/bis-

acryl Long Ranger gels. All sequences have been deposited in GenBank (GenBank accession numbers AF307356–AF307447).

Data Analysis

RAPD bands were scored as presence/absence data, excluding bands that were faint or inconsistent across replicate reactions. All bands scored were between 300 bp and 1600 bp. The presence/absence data matrix was subjected to principal component analysis using SYSTAT ver. 9 (SPSS, Inc., Chicago, IL). This matrix was also converted to a distance matrix using the proportion of difference option in Arlequin ver. 1.1 (Schneider et al. 1997). This measure includes information from both shared presence and absence of bands. A neighbor-joining (NJ) tree was constructed from the distance matrix using the NEIGHBOR program in PHYLIP ver. 3.57c (Felsenstein 1995).

Sequences were aligned using ClustalW ver. 1.7 (Thompson et al. 1994). Minor adjustments were made by eye. Phylogenetic analysis of aligned sequences were conducted using distance (DNADIST and NEIGHBOR) and maximum-likelihood (DNAML) methods in PHYLIP and parsimony using the Winclada/Nona software package (Goloboff 1999; Nixon 1999). For parsimony analysis, a heuristic search was conducted using 100 replications of branch swapping using tree bisection-reconnection with random addition of operational taxonomic units. Characters were unordered and equally weighted. Bootstrap support was calculated for the distance matrix using SEQBOOT in PHYLIP (500 replications) and for parsimony in Winclada/Nona (1000 replications). For maximum-likelihood, the transition:transversion (Tr:Tv) was set to 2. Varying the Tr:Tv ratios made no difference in the

topology or branch lengths of NJ trees, and results reported here are from the distances derived using a Tr:Tv of 2. Results using the Kimura (1980) two-parameter (K2P), correction and the Jin and Nei (1990) distance that assumes a gamma distribution of nucleotide substitution did not differ, so all analyses reported here are based on the K2P distances.

For both RAPD and sequence data, analysis of molecular variance (AMOVA; Excoffier et al. 1992) implemented in Arlequin was used to partition components of variance within and among levels of hierarchic structure under different hypotheses (a priori based on observed topographical and ecological features of the continent and a posteriori based on patterns observed in the data). The outgroup taxa were not used in these analyses. A primary interest was the comparison of levels of structure among geographic regions relative to that among host plant species. This analysis required use of a truncated dataset because only those comparisons in which host-plant species are found sympatrically are relevant. Therefore, only the regions ACP, GC, and CE (abbreviations in Fig. 2) and *Vitis* species *V. vulpina, V. aestivalis,* and *V. cinerea* were used. For this AMOVA, geographic regions were nested within host-plant species. In this analysis significant host-plant effects will appear in the among-host-plant-species component of variance, and may influence the within-geographic-regions component if differentiation onto different hosts was spatially restricted. Significant geographical effects will appear as variance among regions within host-plant species. Within each AMOVA, significance of F-statistics (termed Φ-statistics by Excoffier et al. 1992) was calculated from 1000 random permutations of the data. Population pairwise F_{ST}-values were also calculated and their significance tested by permutation (1000 times). Because RAPDs are dominant markers and their homology is not certain, their F_{ST}-values are best interpreted as the proportion of phenotypic variance partitioned between populations. For sequence data, the mean number of pairwise differences, nucleotide diversities (π; from eq. 10.5 in Nei 1987), and Nm (from the relation for haploid data: $Nm = 1 - F_{ST}/2F_{ST}$ = the number of migrants per generation) were calculated with Arlequin. Values of Nm greater than one have traditionally been taken to indicate that gene flow will swamp genetic drift in the differentiation of populations, and values greater than four indicate a panmictic population (Kimura and Weiss 1964). It is noted here that grape phylloxera may not conform, in some or all regions of its range, to the island model of population structure upon which Nm was based (Wright 1931), but it is used as a rough guide to what historical levels of connection among regions may have been. Only values of Nm for which the associated F_{ST} are very large ($Nm > 4$) or very small ($Nm \ll 1$) are reported, because these are robust to violations of the assumptions (Hutchinson and Templeton 1999).

RESULTS

Substantial RAPD variation was observed among grape phylloxera samples (85% polymorphic bands; i.e., no band at a frequency > 0.95). Polymorphism was less among samples from the SW, NC, NE, and PC (39%, 19%, 39%, and 31% polymorphic bands, respectively; abbreviations in Fig.
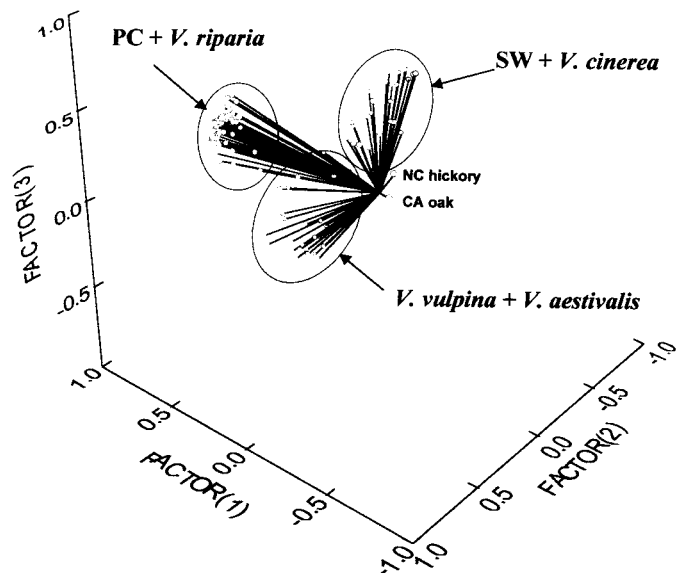

FIG. 3. Principal components plot of RAPD presence/absence data (88 bands). The first three axes explain 65% of the variance in banding patterns. The SW + *V. cinerea* group includes all samples collected in Arizona and New Mexico from *V. arizonica* and all samples collected from *V. cinerea* wherever it was found. The *V. vulpina* + *V. aestivalis* group includes 25 of 26 samples collected from these two hosts wherever they were found. The PC + *V. riparia* group includes all samples collected from vineyards in California, Oregon, Washington, and New York as well as all samples collected from *V. riparia* wherever it was found. NC hickory, *Phylloxera* sp. collected from a *Carya* sp. in North Carolina; CA oak, *Phylloxera* sp. collected from *Quercus lobata* in Davis, California.

2) than among samples from the CE, GC, or ACP (65%, 60%, and 68% respectively). Percent polymorphic bands among samples from *V. vulpina, V. aestivalis, V. cinerea,* and *V. riparia* were 56%, 45%, 56%, and 32%, respectively. Note that data for *V. arizonica* are completely concordant with the region data. The extent of polymorphism reflects the large geographic area sampled, previous RAPD studies found less polymorphism at smaller scales (Lin et al. 1999; Downie 2000).

Principal component analysis distinguished three major groupings (Fig. 3): (1) all samples from the SW and *V. cinerea;* (2) all but one sample from *V. vulpina* and *V. aestivalis;* and (3) all samples taken from *V. riparia* and from vineyards. Thirty-five per cent of the molecular variance was partitioned among these three groups (AMOVA, $P < 0.001$, df = 2) and 45.5% was within subgroups (i.e., within SW, within *V. cinerea,* etc; $P < 0.001$, df = 89). The remaining 19.7% of the variance ($P = 0.03$, df = 3) was distributed between subgroups.

The NJ tree (Fig. 4) shows SW and CE + GC *V. cinerea* samples were clustered together but were differentiated, and ACP *V. cinerea* samples were differentiated from all other grape phylloxera samples. Samples taken from the sympatric hosts *V. vulpina* and *V. aestivalis* were not distinguished, although samples from *V. vulpina* on the ACP were quite divergent from the main cluster of *V. vulpina* and *V. aestivalis* samples. Within a cluster comprised of all *V. riparia* (NC + NE) and vineyard samples, the native and pest samples were differentiated (two exceptions from California vineyards),
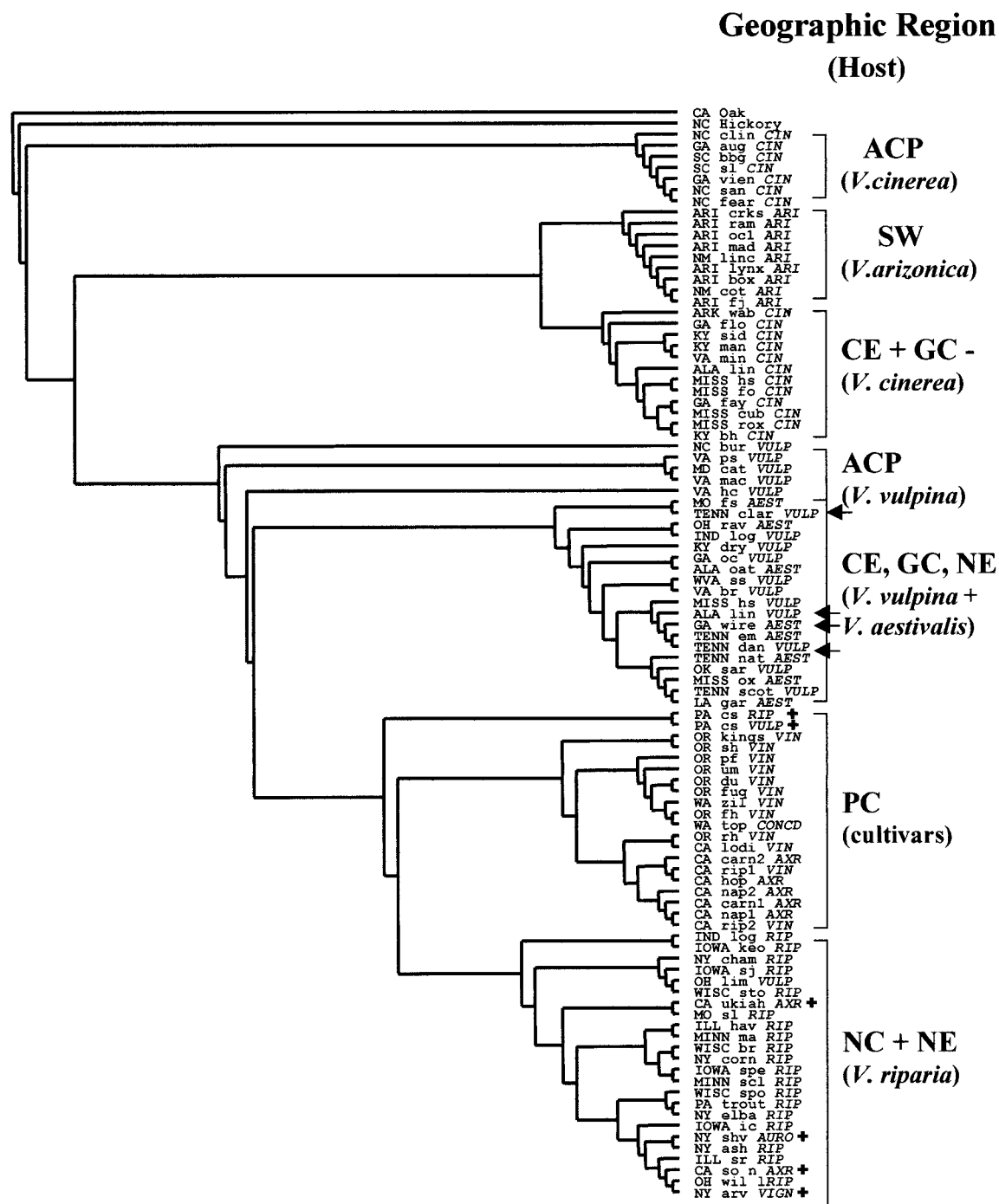
FIG. 4. Neighbor-joining tree of RAPD data. Abbreviations for geographic regions can be found in the caption to Figure 2. Arrows mark samples that appear in the basal grape phylloxera clade in the phylogenetic tree based on COI sequences. Samples collected from vineyards in New York (NYshvAURO, NYarvVIGN) or not consistent with marginal designations (CAukiahAXR, CAsonAXR, PAcsRIP, PAcsVULP) are marked with crosses. The five samples falling outside the main cluster of *Vitis vulpina* and *V. aestivalis* were all collected from *V. vulpina* in North Carolina, Virginia, and Maryland (in the Atlantic Coast–Piedmont [ACP] region).

and Oregon/Washington samples were distinguished from California samples (one exception). Two outliers fell outside this group (PAcsRIP and PAcsVULP). Samples from the New York vineyards were similar in banding patterns to samples from *V. riparia* across the NE + NC. In AMOVA 46.0% (P < 0.001, df = 5) of the variance was distributed between the major clusters in the NJ tree, 7.8% (P < 0.001, df = 5) between putative groupings within these (e.g., *V. cinerea* in the CE vs. *V. cinerea* in the GC), and 46.2% (P < 0.001, df = 85) of the variance was found among samples within such putative groupings.

Analysis of the data partition including only those geo-

TABLE 2. (A) AMOVA results for RAPD data using only those comparisons where *Vitis* species are found sympatrically. The regions considered were the ACP (Atlantic Coast–Piedmont), the CE (Central), and the GC (Gulf Coast; see Fig. 2). The *Vitis* species are *V. cinerea, V. vulpina,* and *V. aestivalis.* (B) Matrix of population pairwise F_{ST}-values (above diagonal) and their *P*-values ($\pm$ SE; below diagonal). Significant values for sympatric comparisons are shown in bold. Values significant after a Bonferroni corrected $\alpha = 0.0018$ are marked with an asterisk. Significance tests for AMOVA and population pairwise F_{ST}-values were conducted by 1000 random permutations of the data. *P*-values represent the proportion of permutations in which a greater component of variance or F_{ST}-values was observed.

A

Source of variation	df	Sum of squares	Variance component	Percentage of variation	P	
Among host plant species	2	137.369	4.395	35.23	0.004	$F_{CT} = 0.352$
Among geographic regions within host plant species	5	60.828	1.056	8.47	<0.0001	$F_{SC} = 0.131$
Within geographic regions	33	231.803	7.024	56.30	<0.0001	$F_{ST} = 0.437$

B

Region-*Vitis* species	ACP-*cinerea*	ACP-*vulpina*	GC-*cinerea*	GC-*vulpina*	GC-*aestivalis*	CE-*cinerea*	CE-*vulpina*	CE-*aestivalis*
ACP-*cinerea*		**0.306**	0.104	0.293	0.411	0.246	0.436	0.450
ACP-*vulpina*	**0.001* (0.001)**		0.408	0.172	0.230	0.530	0.193	0.245
GC-*cinerea*	0.166 (0.011)	0.004 (0.002)		**0.462**	**0.500**	−0.076	0.513	0.530
GC-*vulpina*	0.024 (0.004)	0.041 (0.006)	**0.052 (0.007)**		−0.064	0.668	0.093	0.119
GC-*aestivalis*	0.000* (0.002)	0.015 (0.005)	**0.000* (0.000)**	0.721 (0.013)		0.665	0.014	−0.065
CE-*cinerea*	0.306 (0.004)	0.006 (0.002)	0.965 (0.006)	0.042 (0.006)	0.017 (0.004)		**0.609**	**0.670**
CE-*vulpina*	0.000* (0.000)	0.000* (0.000)	0.001* (0.001)	0.158 (0.011)	0.401 (0.014)	**0.001* (0.001)**		−0.034
CE-*aestivalis*	0.000* (0.000)	0.010 (0.003)	0.019 (0.004)	0.138 (0.011)	0.819 (0.011)	**0.013 (0.004)**	0.737 (0.016)	

graphic regions where *V. vulpina, V. aestivalis,* and *V. cinerea* grow sympatrically suggest that differentiation onto host plant species has occurred but the effect is confounded with geographic structuring (Table 2A). The confounding of host-plant and geographic effects can be teased apart by noting that seven of eight significant population pairwise F_{ST}-values (Bonferroni corrected $\alpha = 0.0018$) involved *V. cinerea,* including three of five possible comparisons involving this host within regions (Table 2B). Thus, the direction of differentiation is onto *V. cinerea.* However, significant F_{ST}-values were observed for ACP versus CE *V. cinerea* and for all comparisons involving ACP *V. vulpina,* suggesting a role for the Appalachian Mountains in restricting gene flow irrespective of host-plant association, possibly combined with the effect of small population size found along the Atlantic Coastal–Piedmont region (Downie et al. 2000).

Downie (2000) found two RAPD markers to have a nonrandom distribution between samples collected from *V. vulpina* and *V. aestivalis* in Missouri, which suggested the possibility that disruptive selection on host use may maintain genetic variation in the manner proposed in Levene-type models (Levene 1953). Only one of these markers (a ~ 320-bp fragment amplified by primer OPG5) was nonrandomly distributed in the current data, and was found at a frequency of 1.0 in samples from *V. aestivalis* (binomial test, *P* = 0.01) and 0.47 (NS) in samples from *V. vulpina.* However, its frequency was also 1.0 in samples from *V. riparia* (*P* < 0.001) and PC (*P* = 0.004), and was 0.0 in SW (*P* < 0.001) and *V. cinerea* (*P* < 0.001) samples, suggesting that the distribution is not a simple reflection of host association.

The RAPD data suggest: (1) an effect of host-plant association on the pattern of molecular variation, attributable to differentiation onto *V. cinerea* with no differentiation between *V. vulpina* and *V. aestivalis;* (2) a close relationship of SW and *V. cinerea* grape phylloxera, consistent with a shared life cycle; and (3) differentiation of grape phylloxera in the

deglaciated region and a close relationship of the pest populations to the natives in this region.

As in other insects (Brown et al. 1994; Funk et al. 1995; Funk 1999; Zimmermann et al. 2000) the COI sequence was strongly AT biased (A = 0.345, T = 0.398, C = 0.153, G = 0.104). The observed Tr:Tv ratio was 1.83 with outgroup sequences, but was 4.78 among grape phylloxera sequences. Pairwise sequence divergence ranged from 7.9% to 13.1% between outgroups and grape phylloxera and from 0.0% to 8.9% within grape phylloxera, and nucleotide diversity was substantial (Table 3). The greatest diversity was observed among grape phylloxera in the GC and CE regions on *V. vulpina* and *V. aestivalis,* whereas very low levels of diversity were observed among samples in the ACP on *V. cinerea* and vineyards in Oregon and Washington.

Of 438 sites aligned unambiguously, 97 were polymorphic, 70 of which were parsimony informative. Fifty-five (79%) of these were third codon position, 14 (20%) were first codon position, and only 1 (1%) was second codon position. Changes at nine of these positions resulted in amino acid substitutions, three of which were changes between grape phylloxera and the outgroups.

NJ, maximum-likelihood, and maximum-parsimony methods recovered similar tree topologies from the COI data. All methods poorly resolved sister group relationships among the majority of grape phylloxera, although many nodes were well supported by bootstrap values and maximum-likelihood confidence limits on branch lengths, indicating significant structure in the data. Figure 5 shows the parsimony-derived majority rule consensus tree. This tree has a length of 474 steps (CI = 0.51, RI = 0.84). At the base of the grape phylloxera clade are four haplotypes collected in the CE or GC regions on *V. vulpina* and *V. aestivalis.* This clade was recovered by all three methods and using different data partitions (one outgroup excluded, just third codon position, just first and second codon positions, data not shown). Sequence diver-

TABLE 3. Nucleotide diversities (the mean number of nucleotide differences per site) and mean number of pairwise differences for mtDNA data based on 438 bp of the COI gene, partitioned according to geographic region and host-plant species. Regionwide values are in bold. Three haplotypes are not included in the regional breakdown, two from cultivated grapes in New York State and one from a single *V. vulpina* vine in the Northeast. ACP, Atlantic Coast–Piedmont; GC, Gulf Coast; CE, Central; NE, Northeast; NC, North-central; SW, Southwest; PC, Pacific Coast.

Region	Host	Nucleotide diversity	Mean no. pairwise differences	n
ACP		**0.017 (0.010)**	**7.49 (3.76)**	**12**
	V. cinerea	0.002 (0.002)	0.88 (0.71)	6
	V. vulpina	0.017 (0.011)	7.43 (4.05)	6
GC		**0.035 (0.019)**	**15.11 (7.33)**	**11**
	V. cinerea	0.014 (0.009)	5.93 (3.40)	5
	V. vulpina	0.067 (0.069)	29.51 (21.22)	2
	V. aestivalis	0.055 (0.037)	23.96 (13.46)	4
CE		**0.036 (0.019)**	**15.61 (7.36)**	**16**
	V. cinerea	0.011 (0.007)	4.63 (2.73)	5
	V. vulpina	0.051 (0.029)	22.25 (11.20)	7
	V. aestivalis	0.020 (0.014)	8.59 (5.04)	4
NE + NC		**0.013 (0.007)**	**5.48 (2.78)**	**16**
	NE-*V. riparia*	0.014 (0.069)	5.97 (3.31)	6
	NC-*V. riparia*	0.012 (0.007)	5.08 (2.69)	10
SW	***V. arizonica***	**0.021 (0.012)**	**9.34 (4.69)**	**11**
PC		**0.025 (0.013)**	**11.03 (5.23)**	**20**
	CA	0.010 (0.006)	4.58 (2.46)	10
	OR/WA	0.002 (0.002)	0.83 (0.64)	10
All *V. vulpina*		**0.045 (0.024)**	**19.65 (9.22)**	**15**
All *V. aestivalis*		**0.037 (0.021)**	**16.26 (8.12)**	**8**
All *V. cinerea*		**0.020 (0.011)**	**8.89 (4.32)**	**16**
All *V. riparia*		**0.013 (0.007)**	**5.48 (2.78)**	**16**
All grape phylloxera		**0.014 (0.020)**	**18.04 (8.08)**	**89**

gence between this clade and its sister group was 2.8%. Southwest grape phylloxera on *V. arizonica* formed a monophyletic clade sister to all other grape phylloxera samples (1.8% sequence divergence from its sister clade), although there were only three base changes (one synapomorphic) along the subtending branch, which was not significant in the maximum-likelihood analysis. Within the SW, a split between samples taken from the Colorado Plateau (ARIoc1ARI, ARIcrksARI, ARIlynxARI, and ARIoc2ARI) and the Sonoran Desert had strong bootstrap support from both parsimony and distance methods and was significant in the maximum-likelihood analysis (1.5% sequence divergence). The remainder of the samples (with one exception) comprised three major clades with equivocal support.

Note that samples taken from the three sympatric host species were distributed throughout the tree. *Vitis vulpina* and *V. aestivalis* samples were placed together in four of five major clades. Consistent with the RAPD data, *V. cinerea* grape phylloxera sampled from ACP formed a well-supported haplotype cluster, as did samples collected in the CE and eastern GC. Samples collected from *V. cinerea* in the Mississippi Basin, however, together with samples from *V. aestivalis* and *V. vulpina* and *V. aestivalis* also collected in the Mississippi Basin, were at the base of a clade consisting of all samples on *V. riparia* taken from the NC and NE (except PAcsRIP), and from Oregon and Washington vineyards (plus one California sample and three *V. vulpina* samples). The *V. riparia*/Oregon/Washington group exhibited low diversity, the majority of haplotypes being either identical or differing by only one or two base changes. No synapomorphies distinguished the Oregon/Washington haplotypes from the native grape phylloxera.

Two haplotypes differing by a single base change were found in nine of 10 California grape phylloxera samples. Two to seven base changes separated the California haplotype cluster from Virginia and Maryland *V. vulpina* samples, whereas six to 10 base changes separated them from all other samples in this clade, suggesting that California phylloxera may be most closely related to *V. vulpina* phylloxera from Virginia or Maryland. Two base changes (one synapomorphic) united the California samples. One sample, CAukiahAXR, was placed in the northern clade, three base changes separated it from its nearest neighbors, and five base changes separated it from the Oregon/Washington haplotypes.

Using AMOVA under different hypotheses of geographic structure, we found that the largest proportion of variance was explained by a structure consisting of the four regions ACP, CE + GC, NE + NC, SW (40.81%, $P < 0.001$, df = 3). AMOVA restricted to the sympatric comparison for the COI data showed that the host-plant effect did not contribute to the pattern of sequence variation, whereas differences among regions within host plant species and within regions made significant contributions to the variance (Table 4A). Significant population pairwise F_{ST}-values in comparisons with *V. cinerea* within regions show that part of the variance within regions may be attributable to differences among hosts (Table 4B).

Estimates of *Nm* ranged from a low of 0.019 between Oregon/Washington and ACP *V. cinerea,* to infinite estimates between the three sympatric host plant species in the GC and CE regions. It is interesting to note the low values of *Nm* obtained for GC and ACP *V. cinerea* (*Nm* = 0.177), CE and ACP *V. cinerea* (*Nm* = 0.122), and ACP *V. vulpina* and ACP *V. cinerea* (*Nm* = 0.340). This suggests that gene flow has
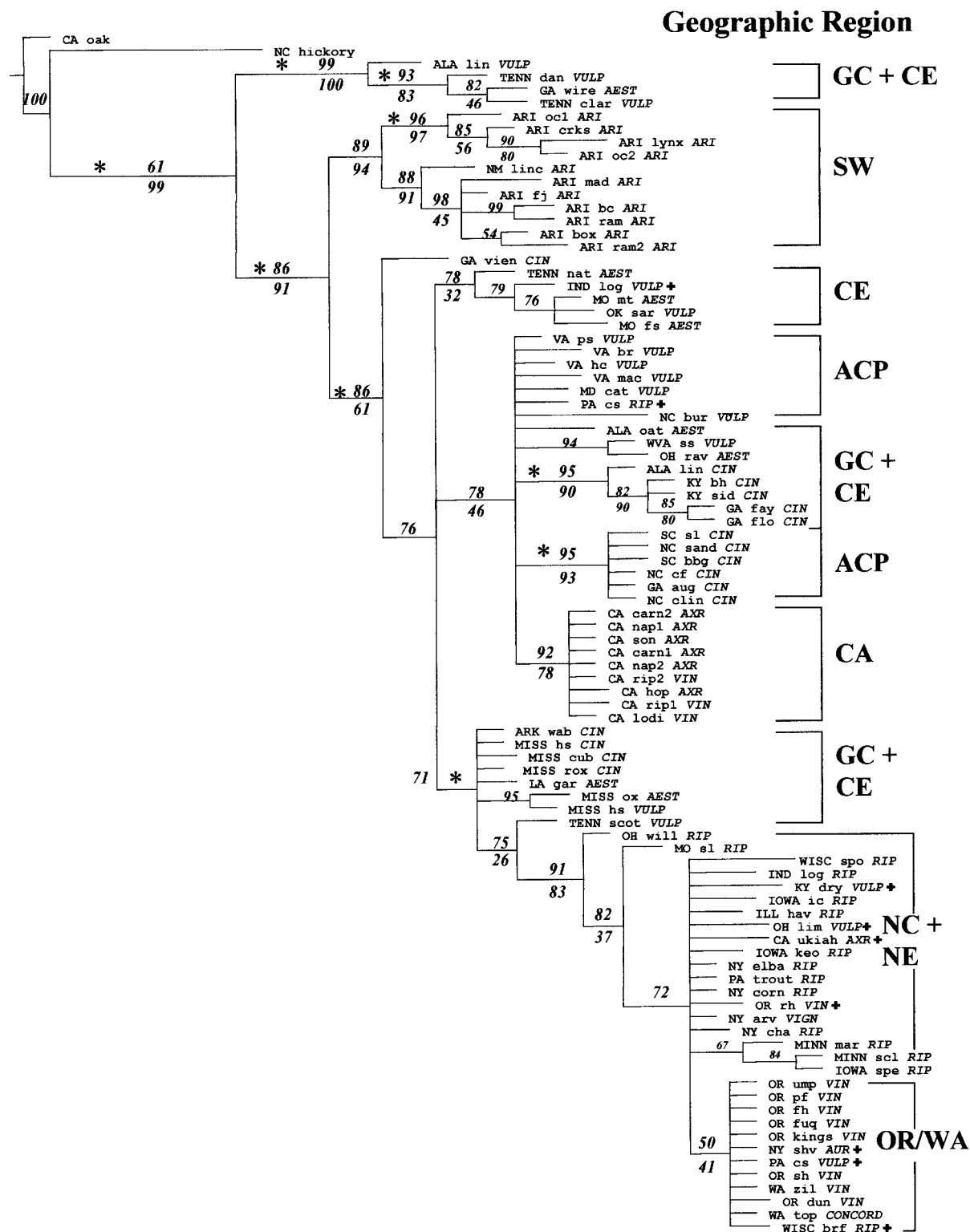
Geographic Region

FIG. 5. Majority rule tree derived from parsimony. A total of 872 equally parsimonious trees were recovered (435 steps, CI = 0.57, RI = 0.87), a consequence of the large number of closely related haplotypes. This tree is shown in lieu of the strict consensus because it agrees with the trees found by neighbor-joining and maximum-likelihood methods. In the strict consensus tree the node separating the SW clade is collapsed. Bootstrap support values are from parsimony above branches (1000 replications) and distance (neighbor joining) below (500 replications). Branches marked with an asterisk have lengths with maximum-likelihood confidence limits that do not overlap zero. Sample codes consist of a state abbreviation, a location abbreviation, and a host plant abbreviation (e.g., GAvienCIN, Georgia, Vienna, *V. cinerea*). Abbreviations for geographic regions can be found in the caption to Figure 2 and sample codes can be matched to locations in the Appendix. Samples that do not conform to the marginal codes are marked with crosses. PAcsRIP and PAcsVULP were collected from the same site and may be miscoded.

TABLE 4. (A) AMOVA results for COI data using only those comparisons where *Vitis* species are found sympatrically. The regions considered were the Atlantic Coast–Piedmont, ACP; Central, CE; and Gulf Coast, GC (see Fig. 2). Because a negative variance component was obtained for the among-host-plant-species factor, the percentage of variation when this is set to zero is given in parentheses. The *Vitis* species are *V. cinerea, V. vulpina,* and *V. aestivalis*. (B) Matrix of population pairwise F_{ST}-values (above diagonal) and their *P*-values ($\pm$ SE; below diagonal). Significant values for sympatric comparisons are shown in bold. Values significant after a Bonferroni corrected $\alpha = 0.0018$ are marked with an asterisk. Significance tests for AMOVA and population pairwise F_{ST}-values were conducted by 1000 random permutations of the data. *P*-values represent the proportion of permutations in which a greater component of variance or F_{ST}-values was observed.

A

Source of variation	df	Sum of squares	Variance component	Percentage of variation	P	
Among host plant species	2	21.101	−0.399	−6.16	0.872	$F_{CT} = -0.062$
Among geographic regions within host plant species	5	74.898	2.166	33.42 (31.49)	0.002	$F_{SC} = 0.315$
Within geographic regions	31	146.145	4.714	72.74 (68.51)	<0.001	$F_{ST} = 0.273$

B

Region-*Vitis* species	ACP-*cinerea*	ACP-*vulpina*	GC-*cinerea*	GC-*vulpina*	GC-*aestivalis*	CE-*cinerea*	CE-*vulpina*	CE-*aestivalis*
ACP-*cinerea*		**0.585**	0.725	0.676	0.465	0.793	0.394	0.691
ACP-*vulpina*	**0.001* (0.001)**		0.367	0.438	0.281	0.362	0.266	0.380
GC-*cinerea*	0.003 (0.002)	0.002 (0.001)		0.189	−0.003	0.297	0.022	0.097
GC-*vulpina*	0.036 (0.006)	0.001* (0.001)	0.250 (0.011)		−0.304	0.457	−0.224	0.195
GC-*aestivalis*	0.006 (0.003)	0.005 (0.003)	0.537 (0.015)	0.999 (0.001)		0.235	−0.180	−0.002
CE-*cinerea*	0.002 (0.002)	0.001* (0.001)	0.094 (0.010)	0.054 (0.008)	0.028 (0.005)		**0.216**	**0.410**
CE-*vulpina*	0.003 (0.002)	0.001* (0.001)	0.416 (0.016)	0.918 (0.006)	0.999 (0.005)	**0.024 (0.004)**		−0.001
CE-*aestivalis*	0.004 (0.002)	0.010 (0.003)	0.131 (0.010)	0.217 (0.011)	0.494 (0.020)	**0.002 (0.004)**	0.385 (0.0176)	

been restricted across the Appalachian Mountains, and between the northern and southern Atlantic Coastal–Piedmont region, although the latter comparison confounds host plant and geography. Estimates involving the SW populations ranged from 0.118 to 0.718 ($F_{ST} = 0.48$–0.89). Between the NE and NC regions *Nm* was estimated at 15.216 ($F_{ST} = 0.032$, $P = 0.264$). The smallest F_{ST} (0.24, $P = 0.001$) involving California grape phylloxera was with ACP *V. vulpina,* supporting an inference of close relationship between these phylloxera populations.

DISCUSSION

The paraphyletic relationships of insects collected from different host-plant species in sympatry suggest that divergent selection acting through host-plant traits has not been a primary influence in the diversification of grape phylloxera. Because paraphyly might be expected, however, even if host associated divergence had occurred, its role cannot be discounted completely. The RAPD data differentiate grape phylloxera on *V. cinerea* from other grape phylloxera, including those on sympatric hosts, and further suggest that there are two distinct populations associated with this host, on either side of the Appalachian Mountains. The COI and RAPD data were consistent with respect to *V. cinerea* phylloxera from the Atlantic Coast–Piedmont region (possibly a widely dispersed clonal lineage, or bottlenecked population), but COI sequences failed to place other phylloxera associated with this host in a monophyletic clade. This, and large values of *Nm,* suggest recent gene flow between phylloxera on *V. vulpina, V. aestivalis,* and *V. cinerea* in the central and Gulf Coast regions. Given evidence for differentiation onto *V. cinerea,* the question is whether paraphyly and high *Nm*-values are consequences of recurrent gene flow, incomplete lineage sorting, or secondary contact (see below). Much of the geographic structure in the data arises from peripheral popula-

tions where small N_e and inbreeding due to absence of dispersal of sexuales shortens the time to monophyly (SW and ACP-*V. cinerea*), or where founder effects of recent colonization and expansion will lead to monophyletic clades (NC + NE; see below). This pattern is consistent with a peripheral isolates view of species cladogenesis. Population differentiation onto the sympatric host species *V. vulpina* and *V. aestivalis* has not occurred in spite of a long history of association. The high estimates for *Nm* suggest that the populations on these hosts are panmictic. For the predominantly neutral markers used here differentiation of grape phylloxera on *V. arizonica* and *V. riparia* has likely resulted from geographic shifts rather than host-plant shifts. Whether selection through divergent host-plant traits has contributed to adaptive differentiation in these populations can only be inferred from transfer experiments that measure fitness or its components. It would be revealing to conduct transfer experiments between *V. riparia* and *V. vulpina*. These account for most of the native grape phylloxera population in the eastern United States, and genetic differentiation of phylloxera on *V. riparia* for the most part appears to be maintained though studies in aphids have shown a persistent pattern of movement from south to north mediated by summer southerlies (Irwin and Thresh 1988). A greenhouse experiment showed that Arizona grape phylloxera were unable to gall *V. riparia* genotypes tested (Downie 1999b), but other transfer experiments have not been done across different wild grape species. Molecular analysis from a more detailed sampling along transects through the zone of overlap would be helpful as well.

Differences in life cycle, geographic distribution, and RAPD and AFLP markers have suggested that southwestern grape phylloxera are highly divergent from other grape phylloxera (Lin et al. 1999; Downie and Granett 2000; H. Lin, unpubl. data). In this study, the SW population was monophyletic with both nuclear and mitochondrial markers, but

was not more divergent than some other clades. How far along the continuum of divergence toward species recognition is far enough is a difficult question that we will not attempt to resolve here (Neigel and Avise 1986; de Quieroz 1999). Mating experiments will be extremely difficult to perform because of the requirements for induction of alates, viable sexuales, and diapause in fertilized eggs in the laboratory. Speciation in an allopatric mode may be well underway in the Southwest.

The division into geographic regions used here for heuristic purposes was a poor predictor of genetic structure (21.9% of the RAPD and 23.8% of the mtDNA variance among regions). A better hypothesis of geographic structure would include four regions: (1) the Southwest, split from eastern populations by the arid high plains of the midcontinent; (2) the entire deglaciated region north of the Missouri and Ohio Rivers; (3) the region south of these rivers to the Gulf Coast and west of the Appalachian Mountains; and (4) the region east of the Appalachian Mountains. The parsimony tree and observed levels of nucleotide diversity suggest an origin in the central or Gulf Coast regions of the United States. The low diversity and large clade area observed in the north, on *V. riparia,* argue against a multiple source or broad front of expansion from the south, but suggest that the colonists originated from a fairly restricted source, possibly in the Mississippi Valley. Hewitt (1996, 2000) has suggested that low genetic diversity in formerly glaciated regions could be a consequence of the rapid colonization of these regions by few long-distance dispersers that rapidly fill the space, even though genetic variation may be high in the source populations. It is of interest that the greatest abundance of grape phylloxera is found in the formerly glaciated regions (Downie et al. 2000), a pattern that might be expected from rapid expansion into a recently opened habitat. These results are consistent with those from numerous other studies across a range of taxa (reviewed in Hewitt 1996, 2000; Bernatchez and Wilson 1998).

Concordance of results across different genes, gene regions, genomes, and methods has been a topic of considerable interest (Palumbi and Baker 1994; Slade et al. 1994; Allendorf and Seeb 2000). The discrepancies in the RAPD and COI data in this study, especially regarding *V. cinerea* grape phylloxera, may imply something other than the fact that different methods may be better or worse at uncovering relationships. For example, it is difficult to see how the pattern observed in Figures 3 and 4 could be the result of RAPD artifacts, because these would be expected to destroy a coherent pattern. If one accepts the premise that RAPDs are a largely random sample of the nuclear genome (Welsh and McClelland 1990; Williams et al. 1990) and the theoretical expectation that neutral nuclear loci have older coalescent times that mitochondrial loci (up to fourfold for the case of strict neutrality; Hudson 1990; Avise 2000), then the pattern observed in the RAPD data may reflect an older and the mtDNA a more recent history. Usually the rapid lineage sorting of mtDNA markers is thought to produce better-resolved gene trees (Moore 1995; Avise 2000). This expectation may not be met in nonequilibrium populations or where evolution is reticulate (Moore 1995). Shifting host-plant distributions, for instance, will alter the pattern of gene flow over time. *Vitis cinerea* has a somewhat more southerly distribution than the other *Vitis* species that host grape phylloxera and could have been parapatrically or allopatrically distributed at some time in the past. The truncated life cycle observed in southwestern and *V. cinerea* phylloxera could have evolved in a parapatric or allopatric population that subsequently split into the Southwest and onto *V. cinerea* in the east. More recent contact of populations on *V. cinerea* with populations on *V. vulpina* and *V. aestivalis* has resulted in maternal gene flow from their populations into *V. cinerea* populations. This hypothesis is consistent with the cycles of vicariance that Vrba (1995) suggested would continually renew reproductive connections among populations that undergo temporary phases of allopatric divergence. It would be supported if a tree constructed from nuclear sequences revealed a sister group relationship of *V. cinerea* and southwestern grape phylloxera. A further suggestion in the pattern observed is that a partial reproductive isolation exists through males. It is possible, of course, that the reverse situation holds. Incomplete lineage sorting could cause paraphyly of populations on *V. cinerea* at mtDNA loci. Then RAPD loci would have to be from more rapidly evolving regions, such as short tandem repeats, to explain the observed differences. Hoelzer (1997) suggested that nuclear loci may coalesce more recently than mtDNA when the sex ratio is strongly female biased or when females are philopatric. Where examined the sex ratio in grape phylloxera is 1:1 (Downie and Granett 1998) and all movement is through females (who carry males with them). The RAPD loci may be affected by the selective influences on the life cycle that southwestern and *V. cinerea* phylloxera appear to share, but it is difficult to see how selection on this trait could register across as many arbitrarily located sequences as were found in these data.

Based on the mtDNA data we are limited to the suggestion that the life cycle shared by grape phylloxera in the Southwest and on *V. cinerea* either evolved independently or is a plastic response to environmental stimuli, common in aphids and their relatives (Hales et al. 1997). In the present case, a plastic response would have to be mediated directly through the host plant because the life cycle variants have been observed on co-existing host-plant species within the same habitats, across a broad geographic expanse (from Louisiana to Virginia). They have been observed to be stable in greenhouse and in vitro environments as well (D. A. Downie, Forneck et al. 2001; unpubl. data).

Substantial genetic diversity and phylogeographic structure was found in grape phylloxera using a conserved region (5′ end) of a conserved gene (COI). However, clear evidence for speciation was not found. There are three factors to consider. First, extensive gene flow on a broad scale is ongoing, despite the apparent sedentary life history of phylloxera. The high *Nm*-values among GC and CE populations certainly support this. Such gene flow could be driven by wind-blown dispersal of crawlers and alates and random colonization of new host plants. The pattern of gene flow over time may have been strongly affected by shifting distributions of host plant species under the influence of glacial advance and recession, as discussed above with reference to *V. cinerea.* Second, lineage extinction could prevent sustained divergence and erase much of the evolutionary history. Lineage extinction could involve the stochastic loss of mitochondrial lineages, as well

as demographic extinction/colonization dynamics. An extinction rate of colonies of grape phylloxera of 0.23/year/vine has been observed in the southwestern United States (Downie and Granett 1999), and it is possible that this type of dynamic scales up to local and even regional populations at longer time scales. The four samples at the base of the COI tree seem to indicate this type of effect, and may be the remnant of a once more widespread mitochondrial lineage. If these samples had by chance not been collected (i.e., were extinct or at even lower frequency), one might be more inclined to invoke a cladogenetic event resulting in a new species of grape phylloxera in the southwestern United States. Finally, grape phylloxera may have a recent origin. Because speciation is expected to progress through a continuum where sequences will be polyphyletic, paraphyletic, and finally reciprocally monophyletic (Neigel and Avise 1986), knowledge of the age of a group will improve the predictive power of hypotheses regarding constituent populations. A molecular phylogeny for the family Phylloxeridae could resolve this problem for grape phyloxera.

We recognize that the sampling scheme used here ignores fine-scale structure (among vines and host species within sites and among sites within regions), focusing instead on broad-scale patterns (within and among host species and geographic regions). The consequences of neglecting variation within and among sites would be negligible if such variation was absent or would tend to overestimate differentiation among geographic regions if such variation was high. It would not, however, alter the conclusions drawn here about differentiation onto host plant species unless such differentiation was a quite geographically restricted phenomenon. This possibility therefore remains open. Previously, for RAPD markers at least, very low levels of variation were found within local populations in Arizona and New York (Lin et al. 1999), suggesting the strategy used here is justified, but higher levels of variation were found in Missouri, which was structured among individual vines (Downie 2000). This variation did not however, correlate with host plant species association, and is consistent with the large variance within regions in AMOVA observed in this study.

Grape phylloxera introduced to Oregon and Washington apparently originated on *V. riparia* in the northern United States; similarity among haplotypes makes it difficult to pinpoint a more precise geographic location. Most (but not all) California grape phylloxera are probably derived from a different region (ACP) and the data suggest a *V. vulpina* origin. Although there have clearly been at least two introductions into viticulture on the Pacific Coast (and within California), there may not have been many more. Management strategies that utilize host-plant resistance derived from North American grape species may need to differ in these states. Although heterogeneity in deployment of host-plant resistance is common (Gould 1983), our evidence regarding the ancestral hosts and geographic regions is new and potentially useful for efforts to locate resistant germplasm.

The results are of interest in the evolutionary interpretation of biotypes. A previous RAPD study (Fong et al. 1995) found that clones phenotypically classified as biotype A or biotype B were not distinguished. If we assume that the host of collection equates with biotypic phenotype (biotype B from

AXR 1, biotype A from *V. vinifera*), then the current RAPD data are consistent with those of Fong et al. (Fig. 4). The COI data suggest that the phenotypes biotype A and biotype B are phylogenetically indistinguishable (Fig. 5). Two nearly identical haplotypes were observed in nine of 10 California samples (six from AXR 1, three from *V. vinifera*). The prediction that little divergence in molecular sequence would be observed had there been a single introduction with subsequent adaptive differentiation was borne out, in part. The placement of one sample (CAukiahAXR collected from AXR 1) in the northern clade is evidence, however, that insects categorized as biotype B have independent histories and have converged in their pattern of host-plant use. The data do not point to a preagricultural origin of differentiated pest populations, as has been suggested for some other insects (Porter et al. 1997; Shufran et al. 2000), in which case samples from the different cultivars would have appeared in separate clades. The implication of these observations is that most California insects derive from a common mitochondrial lineage, but are polymorphic at nuclear loci conditioning host use—insects distinguished by patterns of host use on cultivated vines could have been sampled from a single native host in a restricted area. Alleles affecting fitness on different hosts and segregating at nuclear loci in the local population from which they were originally (accidentally) sampled (but present elsewhere as well) may, in the asexual population in California, be frozen in genotypes with different nuclear backgrounds. Clonal selection could increase the frequency of clones with genotypes favored in the current environment of host-plant resistance (but may otherwise be unrelated). The set of clones that are classified under a biotype name may not share a common ancestor and cannot become cohesive through recombination. A characterization of the genetic basis of host use, although difficult in an asexual organism, will be more constructive for the future than enumerating biotypes.

In conclusion, this study documents a distribution of genetic variation that favors geographic influences, although not exclusively. Evidence for divergence in sympatry on the grape species *V. cinerea* was equivocal but cannot be ruled out. Ongoing gene flow, lineage extinction, stochastic dispersal, and shifting host-plant distributions could all prevent sustained divergence. Linkage of host choice and host-associated mating has been the cornerstone of the sympatric model of speciation developed by Bush and coworkers (Maynard Smith 1966; Diehl and Bush 1989; Johnson et al. 1996). Host choice has been demonstrated in grape phylloxera but only at a fine spatial scale (Omer et al. 1999) and is not likely to operate at large spatial scales. Frequent stochastic colonization may erode the potential for the linkage of host-choice/host-associated mating and forestall divergence across broadly distributed host plants. Finally, there appear to be at least two sources of introductions into viticulture on the Pacific Coast, and a polyphyletic origin of biotype B. Part of the difficulty in dissecting the genetic basis of host use in grape phylloxera (as well as other pests) stems from the asexual mode of reproduction in the pest populations and in part from adherence to a loosely applied and ambiguous biotype concept. Too often, named biotypes are founded on insufficient data to infer host race formation and their categorization is distracting to more fruitful lines of research (Claridge and

Den Hollander 1983). In addition, if our interest is in the processes that have generated the biological diversity observed in nature, the formation of host races as a consequence of the manipulation of the environment by humans is not of obvious relevance. However, the course of evolution is now being irrevocably affected by human manipulations, and our study must include this newer act of the evolutionary play.

In future work we will attempt to better resolve relationships among grape phylloxera populations by acquiring additional sequence data from both mitochondrial and nuclear loci, trace the ancestry of grape phylloxera in the introduced populations globally, and generate a phylogeny for the family Phylloxeridae.

LITERATURE CITED

Abrahamson, W. G., and A. E. Weis. 1997. Evolutionary ecology across three trophic levels. Princeton Univ. Press, Princeton, NJ.

Allendorf, F. W., and L. W. Seeb. 2000. Concordance of genetic divergence among sockeye salmon populations at allozyme, nuclear DNA, and mitochondrial DNA markers. Evolution 54: 640–651.

Allmon, W. D. 1992. A causal analysis of stages in allopatric speciation. Pp. 219–257 in D. Futuyma and J. Antonovics, eds. Oxford surveys in evolutionary biology. Oxford Univ. Press, Oxford, U.K.

Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. J. Mol. Biol. 215: 403–410.

Avise, J. C. 2000. Phylogeography. Harvard Univ. Press, Cambridge, MA.

Bailey L. H. 1934. The species of grapes peculiar to North America. Gentes. Herb. 3:150–244.

Barraclough, T. G., and A. P. Vogler. 2000. Detecting the geographical pattern of speciation from species-level phylogenies. Am. Nat. 155:419–434.

Barton, N., and B. Charlesworth. 1984. Genetic revolutions, founder effects, and speciation. Annu. Rev. Ecol. Sys. 15:133–164.

Berlocher, S. H. 1998. Can sympatric speciation via host or habitat shift be proven from phylogenetic and biogeographic evidence? Pp. 99–113 in D. J. Howard and S. H. Berlocher, eds. Endless forms. Oxford Univ. Press, New York.

Bernatchez, L., and C. C. Wilson. 1998. Comparative phylogeography of nearctic and palearctic fishes. Mol. Ecol. 7:431–452.

Brown, J. M., O. Pellmyr, J. N. Thompson, and R. G. Harrison. 1994. Phylogeny of *Greya* based on nucleotide sequence variation in mitochondrial cytochrome oxidase I and II: congruence with morphological data. Mol. Biol. Evol. 11:128–141.

Bush, G. L. 1969. Sympatric host race formation and speciation in frugivorous flies of the genus *Rhagoletis*. Evolution 23:237–251.

———. 1993. Host race formation and speciation in *Rhagoletis* fruit flies. Psyche 99:335–357.

Carson, H. L. 1982. Speciation as a major reorganization of polygenic balances. Pp. 411–433 in C. Barigozzi, ed. Mechanisms of speciation. A. R. Liss, New York.

Claridge, M. L., and J. Den Hollander. 1983. The biotype concept and its application to insect pests of agriculture. Crop Prot. 2: 85–95.

Davidson, W. M., and R. L. Nougaret. 1921. The grape phylloxera in California. U.S. Dep. Agric. Bull. 903:1–127.

De Benedictis, J. A., and J. Granett. 1992. Variability of responses of grape phylloxera to bioassays that discriminate between California biotypes. J. Econ. Entomol. 85:1527–1534.

de Quieroz, K. 1999. The general lineage concept of species and the defining properties of the species category. Pp. 49–89 in R. A. Wilson, ed. Species: new interdisciplinary essays. MIT Press, Cambridge, MA.

Diehl, S. R., and G. L. Bush. 1984. An evolutionary and applied perspective of insect biotypes. Annu. Rev. Entomol. 29: 471–504.

———. 1989. The role of habitat preference in adaptation and speciation. Pp. 345–365 in D. Otte and J. A. Endler eds. Speciation and its consequences. Sinauer Associates, Sunderland, MA.

Dobzansky, T. 1937. Genetics and the origin of species. Columbia Univ. Press, New York.

Downie, D. A. 1999a. Performance of native grape phylloxera on host plants within and among terrestrial islands in Arizona, USA. Oecologia 121:527–536.

———. 1999b. Population structure in native grape phylloxera. Ph.D. diss., University of California, Davis.

———. 2000. Patterns of genetic variation in native grape phylloxera on two sympatric host species. Mol. Ecol. 9:505–514.

Downie, D. A., and J. Granett. 1998. A life cycle variation in grape phylloxera. Southwest. Entomol. 23:11–16.

———. 1999. Distribution, abundance, and short-term persistence of grape phylloxera in two regions of the native range. Environ. Entomol. 28:1004–1013.

———. 2000. Genetic divergence in geographically isolated populations of native grape phylloxera. Southwest. Entomol. 25: 255–264.

Downie, D. A., J. Granett, and J. R. Fisher. 2000. Distribution and abundance of leaf galling grape phylloxera and *Vitis* species in the central and eastern United States. Environ. Entomol. 29: 979–986.

Excoffier, L., P. E. Smouse, and J. Quattro. 1992. Analysis of molecular variance from metric distance among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491.

Felsenstein, J. 1995. PHYLIP: phylogeny inference package. Ver. 3.5. Dept. of Genetics, Univ. of Washington, Seattle, WA.

Fergusson-Kolmes, L. A., and T. J. Dennehy. 1993. Differences in host utilization by populations of North American grape phylloxera. J. Econ. Entomol. 86:1502–1511.

Fong, G., M. A. Walker, and J. Granett. 1995. RAPD assessment of California phylloxera diversity. Mol. Ecol. 4:459–464.

Forneck, A., Y. Jin, A. Walker, and R. Blaich. 1999. Karyotype studies on grape phylloxera. Vitis 38:123–125.

Forneck, A., M. A. Walker, and R. Blaich. 2001. An in vitro assessment of phylloxera life cycle. J. Appl. Entomol. In press.

Funk, D. J. 1999. Molecular systematics of cytochrome oxidase I and 16S from *Neochlamisus* leaf beetles and the importance of sampling. Mol. Biol. Evol. 16:37–82.

Funk, D. J., D. J. Futuyma, G. Orti, and A. Meyer. 1995. Mitochondrial DNA sequences and multiple data sets: a phylogenetic study of phytophagous beetles. Mol. Biol. Evol. 12:627–640.

Futuyma, D. J., and G. C. Mayer. 1980. Non-allopatric speciation in animals. Syst. Zool. 29:254–271.

Goloboff, P. A. 1999. Nona. Ver. 2.0. Fundación e Instituto Miguel Lillo, de Tucumán, Argentina.

Gould, F. 1983. Genetics of plant-herbivore systems: interactions between applied and basic study. Pp. 599–653 in R. F. Denno and M. S. McClure, eds. Variable plants and herbivores in natural and managed systems. Academic Press, New York.

Granett, J., B. Bisabri-Ershadi, and J. Carey. 1983. Life tables of

phylloxera on resistant and susceptible grape roots. Entomol. Exp. Applic. 34:13–19.

Granett, J., P. Timper, and L. A. Lider. 1985. Grape phylloxera biotypes in California. J. Econ. Ent. 78:1463–1467.

Granett, J., J. A. De Benedicitis, J. A. Wolpert, E. Weber, and A. C. Goheen. 1991. Deadly insect pest poses increased risk to north coast vineyards. Calif. Agric. 45:30–32.

Hales, D. F., J. Tomiuk, K. Wohrman, and P. Sunnucks. 1997. Evolutionary and genetic aspects of aphid biology: a review. Eur. J. Entomol. 94:1–55.

Hawthorne, D. J., and T. J. Dennehy. 1991. Reciprocal movement of grape phylloxera alates and crawlers between two differentially phylloxera-resistant grape cultivars. J. Econ. Entomol. 70: 63–76.

Hawthorne, D. J., and S. Via. 1994. Variation in performance on two grape cultivars within and among populations of grape phylloxera from wild and cultivated habitats. Entomol. Exp. Appl. 70:63–76.

Hewitt, G. M. 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. Biol. J. Linn. Soc. 58: 247–276.

———. 2000. The genetic legacy of the Quaternary ice ages. Nature 405:907–913.

Hoelzer, G. A. 1997. Inferring phylogenies from mtDNA variation: mitochondrial gene-trees versus nuclear gene-trees revisited. Evolution 51:622–626.

Howard, D. J., and S. H. Berlocher. 1998. Endless forms. Oxford Univ. Press, New York.

Hudson, R. R. 1990. Gene genealogies and the coalescent process. Oxf. Surv. Evol. Biol. 7:1–44.

Hutchison, D. W., and A. R. Templeton. 1999. Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. Evolution 53:1898–1914.

Irwin, M. E., and J. M. Thresh. 1988. Long-range aerial dispersal of cereal aphids as virus vectors in North America. Phil. Trans. R. Soc. Lond. B 321:421–446.

Jin, L., and M. Nei. 1990. Limitations of the evolutionary parsimony method of phylogenetic analysis. Mol. Biol. Evol. 7:82–102.

Johnson, P. A., F. C. Hoppensteadt, J. J. Smith, and G. Bush. 1996. Conditions for sympatric speciation: a diploid model incorporating habitat fidelity and nonhabitat assortative mating. Evol. Ecol. 10:187–205.

Kawecki, T. J. 1996. Sympatric speciation driven by beneficial mutations. Proc. R. Soc. Lond. Ser. B 263:1515–1520.

Kimura, M. 1980. A simple method for estimating rate of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16:111–120.

Kimura, M., and G. H. Weiss. 1964. The stepping stone model of population structure and the decrease of genetic correlation with distance. Genetics 49:561–576.

Kondrashov, A. S., and M. V. Mina. 1986. Sympatric speciation: when is it possible? Biol. J. Linn. Soc. 27:201–223.

Levene, H. 1953. Genetic equilibrium when more than one niche is available. Am. Nat. 87:331–333.

Lin, H., and M. A. Walker. 1996. Extraction of DNA from eggs of grape phylloxera for use in RAPD testing. Vitis 35:87–89.

Lin, H., D. A. Downie, M. A Walker, J. Granett, and G. English-Loeb. 1999. Genetic structure in native populations of grape phylloxera. Ann. Entomol. Soc. Am. 92:376–381.

Lynch, J. D. 1989. The gauge of speciation: on the frequencies of modes of speciation. Pp. 527–556 in D. Otte and J. A. Endler, eds. Speciation and its consequences. Sinauer Associates, Inc., Sunderland, MA.

Maillet, P. 1957. Contribution a l'etude de la biologie du Phylloxera de la vigne. An. Sci. Nat. Zool. et Biol. Anim. Ser II 19:283–410.

Maynard Smith, J. 1966. Sympatric speciation. Am. Nat. 100: 637–650.

Mayr, E. 1963. Animal species and evolution. Belknap Press, Cambridge, MA.

Menken, S. B. J. 1981. Host races and sympatric speciation in small ermine moths, Yponomeutidae. Entmol. Exp. Appl. 30:280–292.

Moore, M. O. 1991. Classification and systematics of eastern North American Vitis north of Mexico. Sida 14:339–367.

Moore, M. O., and D. E. Giannasi. 1994. Foliar flavonoids of eastern North American Vitis species north of Mexico. Plant Syst. Evol. 193:21–36.

Moore, W. S. 1995. Inferring phylogenies from mtDNA variation: mitochondrial-gene trees versus nuclear-gene trees. Evolution 49:718–726.

Morgan, T. H. 1909. Sex determination and parthenogenesis in phylloxerans and aphids. Science 29:234–237.

Munson, T. V. 1909. The foundations of American grape culture. Munson and Son, Denison, TX.

Nei, M. 1987. Molecular evolutionary genetics. Columbia Univ. Press, New York.

Neigel, J. E., and J. C. Avise. 1986. Phylogenetic relationships of mitochondrial DNA under various demographic models. Pp. 515–534 in S. Karlin and E. Nevo, eds. Evolutionary processes and theory. Academic Press, New York.

Nixon, K. C. 1999. Winclada. Beta ver. 0.9.9. Published by the author, Ithaca, NY.

Omer, A. D., J. Granett, L. Kocsis, and D. A. Downie. 1999. Preference and performance responses of California grape phylloxera to different Vitis rootstocks. J. Appl. Entomol. 123:341–346.

Palumbi, S. R., and C. S. Baker. 1994. Contrasting population structure from nuclear intron sequences and mtDNA of humpback whales. Mol. Biol. Evol. 11:426–435.

Patterson, H. E. 1981. The continuing search for the unknown and unknowable: a critique of contemporary ideas on speciation. S. Afr. J. of Sci. 77:113–119.

Porter, D. R., J. D. Burd, K. A. Shufran, J. A. Webster, and G. L. Teetes. 1997. Greenbug biotypes: selected by resistant cultivars or preadapted opportunists? J. Econ. Entomol. 90:1055–1065.

Rice, W. R. 1987. Selection via habitat specialization: the evolution of reproductive isolation as a correlated character. Evol. Ecol. 38: 1:301–314.

Riley, C. V. 1876. Eighth annual report on the noxious, beneficial, and other insects of the state of Missouri. Pp. 157–168 in Eleventh annual report of the State Board of Agriculture of the State of Missouri. Regan and Carter, Jefferson City, MO.

Rosenzweig, M. L. 1978. Competitive speciation. Biol. J. Linn. Soc. 10:275–289.

Saxena, R. C., and A. A. Barrion. 1987. Biotypes of insect pests of agricultural crops. Insect Sci. Appl. 8:453–458.

Schluter, D. 1996. Ecological speciation in postglacial fishes. Phil. Trans. R. Soc. Lond. B 351:807–814.

Schneider, S., J. Kueffer, D. Roessli, and L. Excoffier. 1997. Arlequin ver. 1.1: a software for population genetic data analysis. Genetics and Biometry Laboratory, Univ. of Geneva, Switzerland.

Shufran, K. A., J. D. Burd, J. A. Anstead, and G. Lushai. 2000. Mitochondrial DNA sequence divergence among greenbug biotypes: evidence for host-adapted races. Insect Mol. Biol. 9: 179–184.

Simon, C., F. Frati, A. Beckenbach, B. Crespi, H. Liu, and P. Flook. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Entomol. Soc. Am. 87: 651–701.

Slade, R. W., C. Moritz, and A. Heideman. 1994. Multiple nuclear-gene phylogenies: application to pinnipeds and comparison with a mitochondrial DNA gene phylogeny. Mol. Biol. Evol. 11: 341–356.

Stoetzel, M. 1985. Pupiform larvae in the Phylloxeridae. Proc. Entomol. Soc. Wash. 87:535–537.

Tauber, C. A., and M. J. Tauber. 1989. Sympatric speciation in insects: perception and perspective. Pp. 307–344 in D. Otte and J. A. Endler, eds. Speciation and its consequences. Sinauer Associates, Inc., Sunderland, MA.

Templeton, A. 1981. Mechanisms of speciation: a population genetic approach. Annu. Rev. Ecol. Sys. 12:23–48.

Thompson J. D., D. G. Higgins, and T. J. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap

penalties and weight matrix choice. Nucleic Acids Res. 22: 4673–4680.

Vrba, E. S. 1995. Species as habitat-specific, complex systems. Pp. 3–44 *in* D. M. Lambert and H. G. Spencer, eds. Speciation and the recognition concept: theory and application. Johns Hopkins Univ. Press, Baltimore, MD.

Wakely, J. 2000. The effects of subdivision on the genetic divergence of populations and species. Evolution 54:1092–1101.

Walsh, B. J. 1864. On phytophagic varieties and phytophagic species. Proc. Entomol. Soc. Philadelphia 3:403–430.

Welsh, J., and M. McClelland. 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. 18:7213–7218.

Williams, J. G., A. R. Kubelik, K. J. Livak, and J. A. Rafalski. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18:6531–6535.

Wood, T. K., and S. I. Guttman. 1983. *Enchenopa binotata* complex: sympatric speciation? Science 220:310–312.

Wright, S. 1931. Evolution in Mendelian populations. Genetics 16: 97–159.

Zimmermann, M., N. Wahlberg, and H. Descimon. 2000. Phylogeny of *Euphydryas* checkerspot butterflies based on mitochondrial DNA sequence data. Ann. Ent. Soc. Am. 93:347–355.

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APPENDIX

Sampling locations for molecular phylogeography of grape phylloxera in the United States. Date, collecting date; leaf/root, whether sample was collected from leaf gall or roots; wild/cult, whether sample was collected from wild grapes or cultivated grapes in vineyard situation; S/P, whether sample consisted of eggs of a single adult or pooled eggs and adults of multiple galls. All samples collected from roots of cultivated grapevines were cloned in the laboratory from single founders. The code shown on trees is in parentheses. All 98 samples were used in RAPD analyses, 92 were used for COI sequences.

Location (code)	Date	Host	Leaf/root	Wild/cult	S/P	Region
Fayetteville, GA (GAfayCIN)	6/4/99	*V. cinerea*	leaf	wild	P	GC
Flovilla, GA (GAfloCIN)	6/4/99	*V. cinerea*	leaf	wild	P	GC
Rt. 23/Ocmulgea River, GA (GAocVULP)	6/4/99	*V. vulpina*	leaf	wild	P	GC
Vienna, GA (GAvienCIN)	6/4/99	*V. cinerea*	leaf	wild	S	GC
Wire Bridge, GA (GAwireAEST)	6/5/99	*V. aestivalis*	leaf	wild	S	GC
Oat Hill, AL (ALAoatAEST)	6/5/99	*V. aestivalis*	leaf	wild	S	GC
Linden, AL (ALAlinCIN)	6/5/99	*V. cinerea*	leaf	wild	P	GC
Linden, AL (ALAlinVULP)	6/5/99	*V. vulpina*	leaf	wild	P	GC
Cuba, MS (MISScubCIN)	6/6/99	*V. cinerea*	leaf	wild	S	GC
Forest, MS (MISSforCIN)	6/6/99	*V. cinerea*	leaf	wild	P	GC
Roxie, MS (MIXXroxCIN)	6/6/99	*V. cinerea*	leaf	wild	P	GC
Gardner, LA (LAgarAEST)	6/7/99	*V. aestivalis*	leaf	wild	P	GC
Sardis Lake, OK (OKsarVULP)	6/8/99	*V. vulpina*	leaf	wild	P	CE
Falling Springs, MO (MOfsAEST)	9/9/97	*V. aestivalis*	leaf	wild	P	CE
Wabbaseka, AR (ARKwabCIN)	6/9/99	*V. cinerea*	leaf	wild	P	CE
Oxford, MS (MISSoxAEST)	6/10/99	*V. aestivalis*	leaf	wild	S	CE
Holly Springs, MS (MISShsCIN)	6/10/99	*V. cinerea*	leaf	wild	P	CE
Holly Springs, MS (MISShsVULP)	6/10/99	*V. vulpina*	leaf	wild	S	CE
Scott's Hill, TN (TENNscotVULP)	6/10/99	*V. vulpina*	leaf	wild	S	CE
Natchez Trace, TN (TENNnatAEST)	6/11/99	*V. aestivalis*	leaf	wild	S	CE
Clarkrange, TN (TENNclarVULP)	6/11/99	*V. vulpina*	leaf	wild	S	CE
Dandridge, TN (TENNdanVULP)	6/11/99	*V. vulpina*	leaf	wild	S	CE
Elk Mills, TN (TENNemAEST)	6/12/99	*V. aestivalis*	leaf	wild	P	CE
Dry Ridge, KY (KYdryVULP)	6/29/99	*V. vulpina*	leaf	wild	P	CE
Big Hill, KY (KYbhCIN)	6/29/99	*V. cinerea*	leaf	wild	S	CE
Manchester, KY (KYmanCIN)	6/29/99	*V. cinerea*	leaf	wild	S	CE
Sidney, KY (KYsidCIN)	6/29/99	*V. cinerea*	leaf	wild	P	CE
Sandstone, WV (WVAssVULP)	6/30/99	*V. vulpina*	leaf	wild	S	CE
Patrick Springs, VA (VApsVULP)	6/12/99	*V. vulpina*	leaf	wild	S	ACP
Burlington, NC (NCburVULP)	6/12/99	*V. vulpina*	leaf	wild	S	ACP
Sanford, NC (NCsanCIN)	6/13/99	*V. cinerea*	leaf	wild	P	ACP
Clinton, NC (NCclinCIN)	6/13/99	*V. cinerea*	leaf	wild	P	ACP
Cape Fear, NC (NCfearCIN)	6/13/99	*V. cinerea*	leaf	wild	S	ACP
Stateline, SC (SCslCIN)	6/13/99	*V. cinerea*	leaf	wild	P	ACP
Batesburg, SC (SCbbgCIN)	6/13/99	*V. cinerea*	leaf	wild	P	ACP
Augusta, GA (GAaugCIN)	6/14/99	*V. cinerea*	leaf	wild	P	ACP
Blue Ridge, VA (VAbrVULP)	6/30/99	*V. vulpina*	leaf	wild	P	ACP
Huff Creek, VA (VAhcVULP)	6/30/99	*V. vulpina*	leaf	wild	S	ACP
Macon, VA (VAmacVULP)	6/30/99	*V. vulpina*	leaf	wild	S	ACP
Mineral, VA (VAminCIN)	6/30/99	*V. cinerea*	leaf	wild	P	ACP
Catoctkin, MD (MDcatVULP)	7/1/99	*V. vulpina*	leaf	wild	P	ACP
Clear Springs Mills, PA (PAcsRIP)	7/1/99	*V. riparia*	leaf	wild	S	NE
Clear Springs Mills, PA (PAcsVULP)	7/1/99	*V. vulpina*	leaf	wild	S	NE
Trout Run, PA (PAtroutRIP)	7/1/99	*V. riparia*	leaf	wild	S	NE
Corning, NY (NYcornRIP)	7/1/99	*V. riparia*	leaf	wild	S	NE
Chambers, NY (NYchamRIP)	7/1/99	*V. riparia*	leaf	wild	S	NE
Geneva, NY (NYarvVIGN	7/2/99	cultivar Vignoles (complex hybrid with *V. vinifera*)	leaf	cult	P	NE
Seneca Falls, NY (NYshvAUR)	7/2/99	cultivar Aurora (complex hybrid with *V. vinifera*)	leaf	cult	S	NE
Elba, NY (NYelbaRIP)	7/2/99	*V. riparia*	leaf	wild	S	NE
Ashford, NY (NYashRIP)	7/2/99	*V. riparia*	leaf	wild	S	NE
Williamsfield, OH (OHwillRIP)	7/3/99	*V. riparia*	leaf	wild	P	NE
Ravenna, OH (OHravAEST)	7/3/99	*V. aestivalis*	leaf	wild	S	NE
Lima, OH (OHlimVULP)	7/3/99	*V. vulpina*	leaf	wild	S	NE
Logansport, IN (INDlogVULP)	7/3/99	*V. vulpina*	leaf	wild	S	NC
Logansport, IN (INDlogRIP)	7/3/99	*V. riparia*	leaf	wild	S	NC
Starved Rock, IL (ILLsrRIP)	7/4/99	*V. riparia*	leaf	wild	S	NC
Stoughton, WI (WISCstoRIP)	7/4/99	*V. riparia*	leaf	wild	S	NC
Black River Falls, WI (WISCbrfRIP)	7/5/99	*V. riparia*	leaf	wild	S	NC

APPENDIX. Continued.

Location (code)	Date	Host	Leaf/root	Wild/cult	S/P	Region
Spooner, WI (WISCspoRIP)	7/5/99	*V. riparia*	leaf	wild	S	NC
St. Cloud, MN (MINNsclRIP)	7/5/99	*V. riparia*	leaf	wild	S	NC
Marshall, MN (MINNmarRIP)	7/6/99	*V. riparia*	leaf	wild	S	NC
Spencer, IA (IOWAspeRIP)	7/6/99	*V. riparia*	leaf	wild	S	NC
St. Joseph, IA (IOWAsjRIP)	7/6/99	*V. riparia*	leaf	wild	S	NC
Iowa City, IA (IOWAicRIP)	7/6/99	*V. riparia*	leaf	wild	S	NC
Keokuk, IA (IOWAkeoRIP)	7/6/99	*V. riparia*	leaf	wild	S	NC
Havanna, IL (ILLhavRIP)	7/7/99	*V. riparia*	leaf	wild	S	NC
Swan Lake, MO (MOslRIP)	9/5/97	*V. riparia*	leaf	wild	P	NC
Oak Creek Canyon, AZ (ARloc1ARI)	1996	*V. arizonica*	leaf	wild	S	SW
Oak Creek Canyon, AZ (ARloc2ARI)	1997	*V. arizonica*	leaf	wild	P	SW
Crooks Creek Canyon, AZ (ARIcrksARI)	1997	*V. arizonica*	leaf	wild	P	SW
Lynx Creek Canyon, AZ (ARIlynx, ARI)	1997	*V. arizonica*	leaf	wild	P	SW
Bear Canyon, AZ (ARIbcARI)	1997	*V. arizonica*	leaf	wild	P	SW
Madera Canyon, AZ (ARImadARI)	1997	*V. arizonica*	leaf	wild	P	SW
Box Canyon, AZ (ARIboxARI)	1997	*V. arizonica*	leaf	wild	P	SW
French Joe Canyon, AZ (ARIfjARI)	1997	*V. arizonica*	leaf	wild	P	SW
Ramsey Canyon, AZ (ARIramARI)	1997	*V. arizonica*	leaf	wild	P	SW
Cottonwood, NM (NMcotARI)	1997	*V. arizonica*	leaf	wild	P	SW
Lincoln, NM (NMlincARI)	1997	*V. arizonica*	leaf	wild	P	SW
Rex Hill, OR (ORrhVIN)	10/6/99	*V. vinifera*	roots	cult	S	PC
Freedom Hills, OR (ORfhVIN)	10/6/99	*V. vinifera*	roots	cult	S	PC
Fuqua, OR (ORfuqVIN)	10/6/99	*V. vinifera*	roots	cult	S	PC
Duncan, OR (ORdunVIN)	10/6/99	*V. vinifera*	roots	cult	S	PC
Salem Hills, OR (ORshVIN)	10/6/99	*V. vinifera*	roots	cult	S	PC
Umpqua, OR (ORumpVIN)	10/6/99	*V. vinifera*	roots	cult	S	PC
Kings Estates, OR (ORkingsVIN)	10/6/99	*V. vinifera*	roots	cult	S	PC
Zillah, WA (WAzilVIN)	10/6/99	*V. vinifera*	roots	cult	S	PC
Toppendish, WA (WAtopCONCORD)	10/6/99	*V. labrusca* cultivar Concord	roots	cult	S	PC
Pfieffer, OR (ORpfVIN)	10/6/99	*V. vinifera*	roots	cult	S	PC
Carneros, CA (CAcarn1AXR)	7/98	rootstock AXR 1	roots	cult	S	PC
Napa, CA (CAnap1AXR)	7/98	rootstock AXR 1	roots	cult	S	PC
Napa, CA (CAnap2AXR)	7/98	rootstock AXR 1	roots	cult	S	PC
Lodi, CA (CAlodiVIN)	7/98	*V. vinifera*	roots	cult	S	PC
Hopland, CA (CAhopAXR)	7/98	rootstock AXR 1	roots	cult	S	PC
Sonoma, CA (CAsonAXR)	7/98	rootstock AXR 1	roots	cult	S	PC
Rippon, CA (CArip1VIN)	7/98	*V. vinifera*	roots	cult	S	PC
Ukiah, CA (CAukiahAXR)	7/98	rootstock AXR 1	roots	cult	S	PC
Rippon, CA (CArip2VIN)	7/98	*V. vinifera*	roots	cult	S	PC
Carneros, CA (CAcarn2AXR)	7/98	rootstock AXR 1	roots	cult	S	PC
Cape Fear, NC (NChicory)	6/13/99	*Carya* sp.	leaf	wild	P	ACP
Davis, CA (CAoak)	9/15/99	*Quercus lobata*	leaf	wild	P	PC